SOME EXPERIMENTS ON SPERMATOGENESIS IN VITRO

By Richard Goldschmidt
(of the Kaiser Wilhelm Institut für Biologie, Berlin)
OSBORN ZOOLOGICAL LABORATORY, YALE UNIVERSITY

Presented to the Academy, March 3, 1915

Unforeseen circumstances that forced me to interrupt my normal work offered a chance for trying some experiments, that I have had in mind for some time, to apply the method of tissue culture to the study of sex-cells with the purpose of rendering the history of these cells accessible to an experimental analysis. The results obtained in this direction during the past winter constitute of course nothing more than the first steps into a very promising field of research. For the experiments the sperm-cells of the moth, Samia cecropia L., were used. The method of cultivation is simply Harrison's method. By tearing the testis of the pupa the thousands of sperm follicles (spermatocysts) are isolated and can easily be mounted in a hanging drop of haemolymph. Naturally the whole procedure must be carried out strictly aseptically. In November most of the follicles contain spermatogonia or young spermatocytes during the growth-period. They could be kept alive in the cultures about three weeks, and during this time a great many follicles finished the process of spermatogenesis up to the full grown spermatozoa under the eye of the observer. The time required depends, as was to be expected, upon the temperature. It seems that the stages from the spermatogonia up to the maturation divisions need weeks for their development but maturation and spermatogenesis occur in a few days even in one day when kept warm. The most striking, although in no way surprising result of the observations is the complete harmony between the facts as observed in the living cell and those described from preserved specimens. The formation of the axial filaments in the young spermatocytes and their subsequent behavior, as well as the mitochondria and their distribution during the maturation divisions, could readily be followed. The interesting and hitherto unobserved behavior of the axial filaments in the formation of the regular sperm-bundles could also be made out. In addition interesting observations were made upon the behavior of the follicle membrane after the death of the sperm cells. At this time an extensive outgrowth of these cells begins, forming a kind of tissue which grows luxuriantly for some weeks more.

The observations made upon normal spermatogenesis suggested the hypothesis that the specific changes undergone by a sperm cell in its development into that strange thread-like spermatozoon may be due

not so much to internal changes inside the cell as to changes in the physical constitution of the surrounding medium, controlled by the follicle membrane, forcing the cell to react in that specific way. In order to test this hypothesis experiments have been made in influencing the osmotic conditions inside the follicular membrane. One of the clearest results was that hypertonic condition in the haemolymph forces all sex-cells from spermatogonia to spermatids to grow out in one direction, namely, against the follicle wall. The resistance of the latter forces the cell to grow along its inner surface so that finally the follicle appears as a whorl of thread-like cells. The amount of growing out depends upon two factors: the age of the cell and the hypertonicity of the medium. Spermatocytes during or before the maturation divisions form long spermatozoa-like threads but with inverse polarity, the axial filament on the inner surface of the cell (present as it is well known in butterflies from the spermatocyte stage on) not being involved at all. The whole process proved to be reversible without change of the medium, as were also the slight changes produced by a hypotonic medium, since after some days all cells returned to their normal form. This means that the follicle membrane has the faculty of active control of the osmotic conditions inside the follicle. In addition to these experiments an effort was made to induce the cells of different age to grow in the normal direction along the axial filaments. Up to the present only the first steps have been obtained by treatment with diluted methyl alcohol. Another normal process that could be induced at a different point in the cell history was the formation of the axial filament. In these experiments interesting observations have been made upon the formation of flagella by the plasm of all sperm cells from the spermatogonia to ripe spermatozoa. They arise in Ringer's Fluid in a similar way as described for other cells by Merk, Kite, Oliver, Chambers, and they can be produced and made to disappear at will by change of temperature.

All these experiments together with the observations of the normal process point to the probability that the general processes of spermatogenesis are necessary reactions of the cells to a systematic regulation of the osmotic conditions on the part of the follicular membrane. The individual specific processes are caused by the specific properties of the reacting cells. This affords us the possibility of an experimental attack upon other closely related problems, viz., the function of Sertoli's cells, which may prove to be the same as the function of the follicle cells in the present case, and the problem of apyrene spermatozoa, which, according to some of my observations, may prove nothing else than a *lusus naturae*, an abnormality produced by slight changes in the physi-

cal properties of the follicle membrane that might readily occur under ordinary conditions. These questions will be discussed in the full account of the work to be published in the *Archiv für Zellforschung*.

GROWTH AND VARIATION IN MAIZE

By Raymond Pearl and Frank M. Surface
BIOLOGICAL LABORATORY, MAINE AGRICULTURAL EXPERIMENT STATION

Presented to the Academy, February 23, 1915

The Problem. The investigation reported in this paper is an attempt to analyze the normal variation of an organism in a particular case, from the standpoint of *Entwicklungsmechanik*. It was suggested in the first instance by the study of variation and differentiation in *Ceratophyllum* made by Pearl some years ago. We have here attempted to approach the problem of *inter*-individual variation from the same point of view and with similar methods to those applied to the problem of *intra*-individual variation in the case of *Ceratophyllum*.

The problem and the point of view may be most clearly defined by considering briefly certain well-known, indeed obvious, facts about variation. If one brings together a homogeneous group of individual plants or animals and measures the same character in each individual, there may be formed from the resulting data a characteristic variation curve for that group and character. The precise form of this curve, as well as the location in it of any particular individual, are functions of two basic variables. Of these one is the hereditary or germinal constitution of the individual. The other is the complex of environmental stresses and strains, which, each acting on the individual during its ontogeny have influenced the end result of the activity of the hereditary determiners or genes.

Now it is altogether usual in discussions of variation and heredity to take the two end terms of the ontogenetic series, the gene on the one hand and the adult soma on the other hand, as things given. What comes between the two is neglected. But clearly what goes between is a part of the very essence of heredity itself.

In any group of adult individuals each one will show some particular variation, in the sense of a deviation from the typical condition of the group. We take it to be one of the final objects of investigations in genetics to find out why (in the sense of locating the essential causal factors involved) a particular individual A exhibits a particular variation a, and not some other variation out of the indefinitely large number